Journal of Biomolecular NMR, **0:** 1–3, 2002.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N NMR assignments of the hypothetical Nudix protein DR0079 from the extremely radiation-resistant bacterium *Deinococcus radiodurans*

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Received 4 November 2002; Accepted 20 November 2002

Key words: Deinococcus radiodurans, DNA maintenance, DNA repair, nudix hydrolase

Biological context

Deinococcus radiodurans is a bacterium that is extremely resistant to the lethal and mutagenic effects of ionizing radiation, ultraviolet radiation, and other physical and chemical DNA-damaging agents (Battista, 1997). It has been suggested that this resistance is due to unusually efficient DNA repair mechanisms (Minton, 1994). Analysis of the complete genome sequence of *D. radiodurans* reveals a full suite of genes with potential DNA repair activities (White et al., 1999), essentially all of which have functional homologues in other procaryotes. These hypothetical DNA repair genes display a high amount of redundancy and include 21 genes that have sequence homology with the Nudix family of polyphosphate pyrophosphohydrolases (Bessman et al., 1996). Nudix proteins are identified by the consensus sequence $GX_5EX_7REUXEEXGU$ (where U = I, L, or V and X = any amino acid) that forms part of the catalytic site for diphosphate hydrolysis (Bessman et al., 1996). Consequently, a nucleoside diphosphate linkage is a feature common in Nudix substrates that include NADH, nucleotide sugars, dinucleotide polyphosphates, and (deoxy)ribonucleoside triphosphates (NTPs). The general biochemical function of the Nudix family of proteins is believed to be sanitizing the cell (Bessman et al., 1996). For example, MutT preferably hydrolyzes the promutagenic NTP 7,8-dihydro-8-oxoguanosine triphosphate to nucleotide monophosphate and inorganic phosphate. Despite the identification of over 450 putative Nudix proteins in genomes on the basis of the Nudix consensus sequence (Gabelli et al., 2001), few Nudix protein structures have been determined (Abeygunawardana et al., 1995; Gabelli et al., 2001; Bailey et al., 2002) and none yet from *D. radiodurans* (Holbrook et al., 2002). To better understand the relevance, function, and mechanism of the Nudix family of proteins, and to better understand the roles played by the hypothetical *D. radiodurans* Nudix proteins in radiation-resistance, we have crystallized the hypothetical *D. radiodurans* Nudix protein DR0079, a 171 residue, 19.3 kDa protein (Holbrook et al., 2002). The ^{1}H, ^{13}C, and ^{15}N chemical shift assignments for DR0079 reported here will enable chemical shift mapping and dynamics studies to be performed on DR0079 using the crystal and/or NMR solution structure of the protein.

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Methods and experiments

The DNA coding sequence for the *D. radiodurans* DR0079 protein was cloned into the vector pET-30b and transfected into the host *Escherichia coli* bacterial strain BL21(DE3) (Novagen, Inc., Madison, WI). Nitrogen-15 and ^{13}C-labeled NMR samples were prepared using minimal media (Adams) containing $^{15}NH_4(SO)_4$ and $^{13}C_6$-glucose supplemented with thiamine (1 μg ml^{-1}) and Fe_2Cl_3 (10 μM). The method for protein induction and purification followed previously described protocols (Holbrook et al., 2002) except for the substitution of the following NMR buffer in the final purification step on a Superdex75

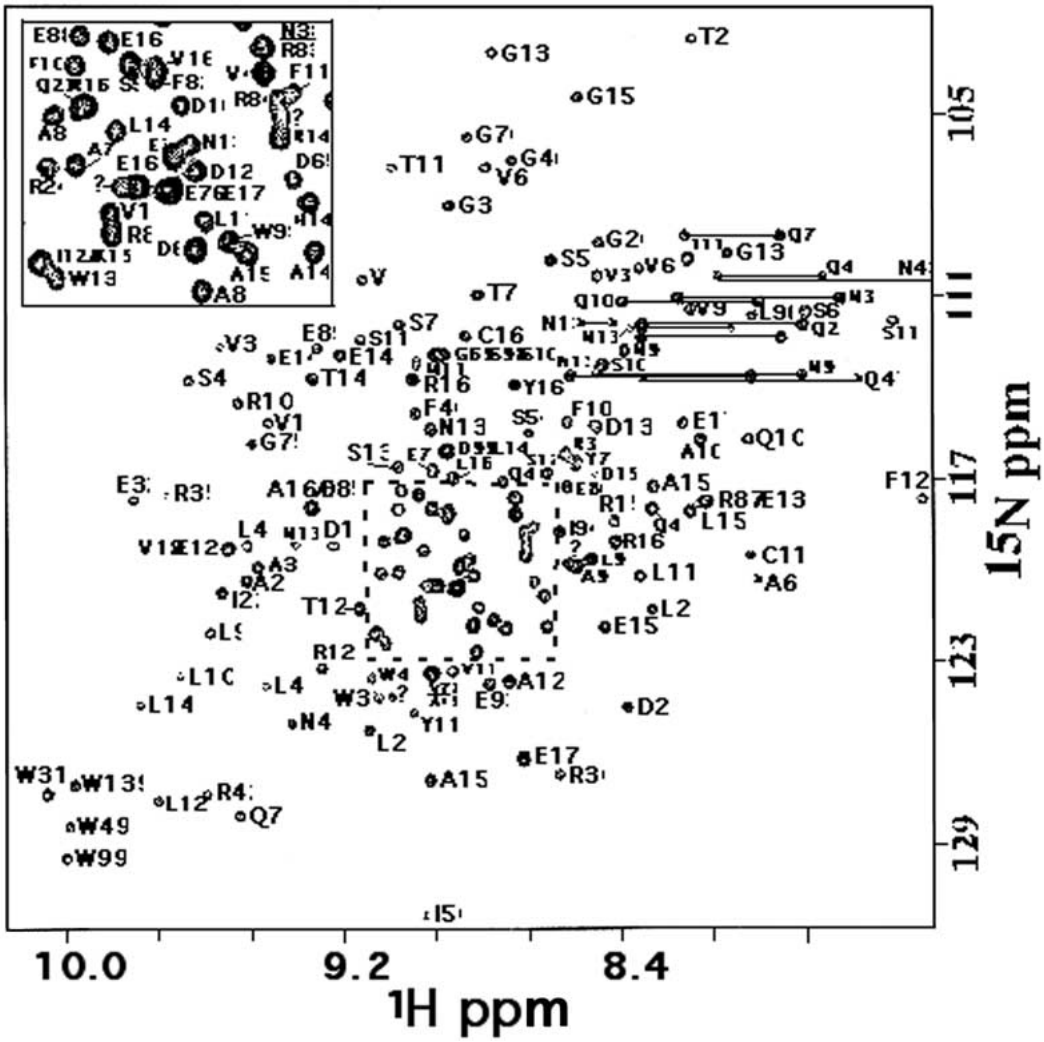

Figure 1. Two-dimensional HSQC spectrum of DR0079 (~2.0 mM, 100 mM KCl, 20 mM potassium phosphate, 2 mM DTT, pH 7.1, 25 °C) collected at a ^{1}H resonance frequency of 800 MHz. Assigned backbone amides are indicated by their residue name and sequence number. Backbone amides that could not be unambiguously assigned are labeled with a question mark (?). Solid horizontal lines connect cross peaks corresponding to amide groups of asparagine and glutamine residues. An (x) marks resonances with intensities below the plotted contour level (A63 backbone amide and N13 and Q47 side chain amides). The inset is an expansion of the congested central region of the spectrum that is outlined with dashed lines.

HiLoad column: 100 mM KCl, 20 mM potassium phosphate, 2 mM DTT, pH 7.1.

Approximately 2.0 and 4.0 mM NMR samples of ^{15}N and ^{13}C-labeled DR0079 were prepared in 250 μL of NMR buffer containing 90% H_2O/10% D_2O and 100% D_2O, respectively. Two-dimensional HSQC and three-dimensional HSQC-NOESY, HNCA, HN-COCA, CBCA(CO)NH, HNCACB, HNCO, HN-CACO, HCCH-TOCSY, CCC-TOCSY-NNH, and CB-CACOCAHA data were collected on the ~2.0 mM sample at 25 °C using Varian 800-, 750- and 600-Inova spectrometers. A four-dimensional CC-NOESY data set was collected on the ~4.0 mM sample on a Varian 600-Inova spectrometer. The methyl groups for 12 of the 13 Val residues and 7 out of the 18 Leu residues were stereospecifically assigned by observing the carbon-carbon splitting of the Pro-R methyl group in the ^{13}C/^{1}H HSQC spectrum of a ~1.0 mM, 10% uniformly ^{13}C-labeled sample (Neri et al., 1989). The data was processed using Felix97 (MSI, San Diego, CA) software. The ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to DSS (DSS = 0 ppm) using indirect methods (Wishart et al., 1995).

Extent of assignment and data deposition

Figure 1 is a ^{15}N/^{1}H HSQC spectrum of DR0079, recorded at a ^{1}H resonance frequency of 800 MHz, labeled with the residue-specific assignments. The good chemical shift dispersion and well defined cross peaks are characteristic of a structured protein in a monomeric state. DR0079 is very robust; the ^{15}N/^{1}H HSQC spectrum of lypophilized protein redissolved in water is identical to the spectrum collected prior to lyophilization. Furthermore, deuterium exchange experiments reveal a subset of 22 cross peaks in the ^{15}N/^{1}H HSQC spectrum that still remain $>$ 2 months after the exchange. In total, 152 out of the 162 possible ^{1}HN resonances (171 residues minus 8 prolines and the terminal amide) were (94%) identified and assigned. For many of these residues complete side chain assignments for the ^{1}H, ^{13}C and ^{15}N resonances have also been made. The assigned ^{1}H, ^{13}C, and ^{15}N chemical shifts for DR0079 are available from the authors as supplementary material and have been deposited in the BioMagResBank in Madison WI, U.S.A. (accession number BMRB-5570).

Acknowledgements

The research was performed in the Environmental Molecular Sciences Laboratory (a national scientific user facility sponsored by the DOE Biological and Environmental Research) located at Pacific Northwest National Laboratory and operated for DOE by Battelle. This work was funded by a grant from the U.S. Department of Energy, Office of Biological Energy Research, Contract No. (DE-AC03-76SF00098).

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